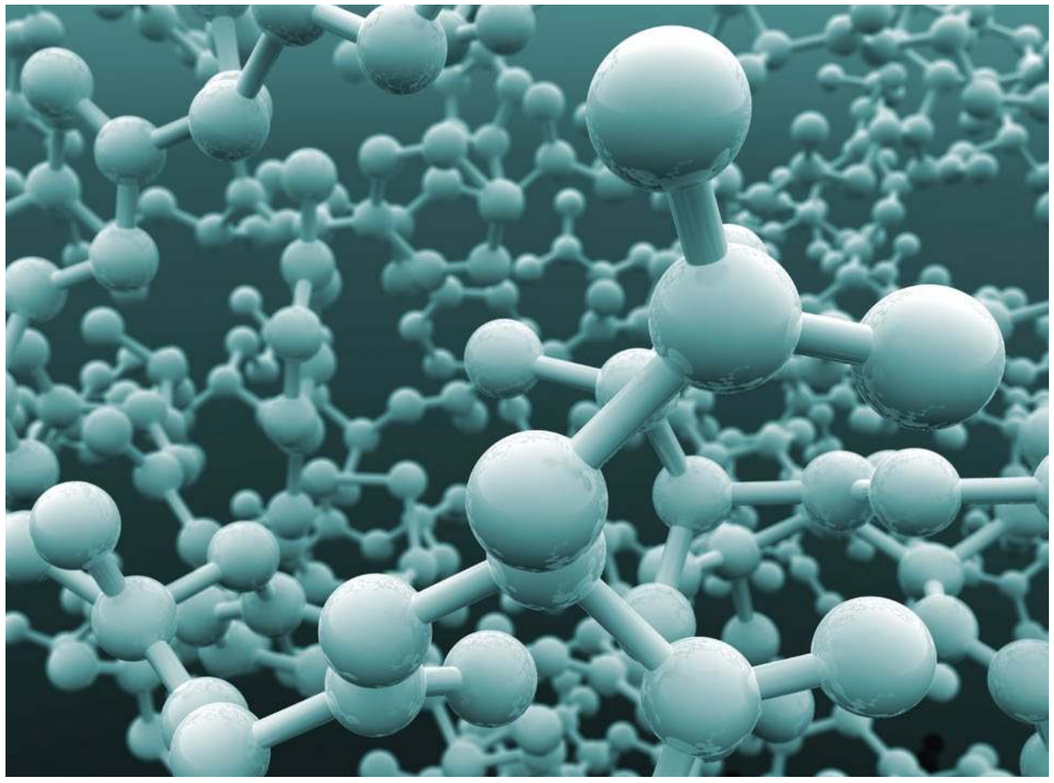
Seeing the structures of molecules:

insights from NMR and industry

Many of the essential chemical constituents of life, including carbohydrates, are made up of complex atomic arrangements. Knowing the particular structure of a compound is important not only for identification purposes, but also for understanding how biologically relevant compounds react. Through this knowledge, their biological functions can ultimately be deciphered. ***Professor Anthony Serianni*** *at the University of Notre Dame has developed a wealth of experimental approaches to understand the 3D structures of such systems, creating widely applicable techniques for structural identification.*

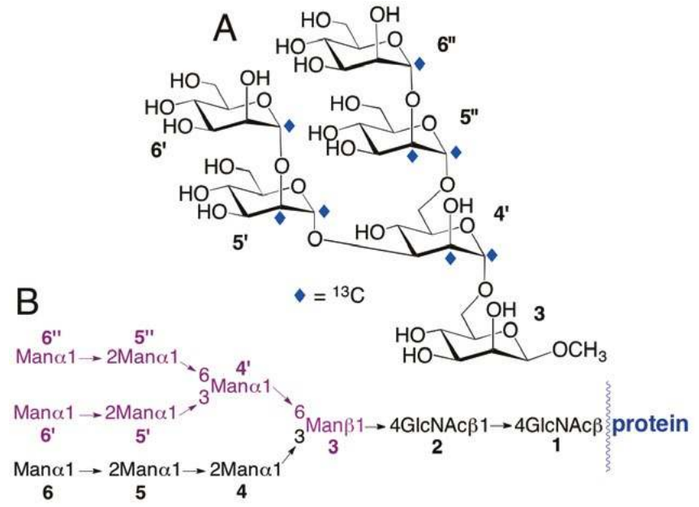


Identifying the structure of molecules remains a key challenge within modern chemistry. In many cases, a molecule's structure has a huge influence on both its chemical and biological activities. For example, carvone, a molecule found in many essential oils and fragrances, can either smell like caraway seeds or spearmint, depending on the orientation of one of the chemical substituents in the molecule. While the two forms of carvone are identical in their atomic composition, it is the physical orientation of one of the groups in the molecule that determines which receptors interact within the human nose and ultimately, how we perceive the smell.

There are still very few experimental techniques capable of identifying molecular structures, particularly in the solution phase. X-ray crystallography is probably the most famous structural identification technique, with the 1964 and 1962 Nobel Prizes in Chemistry both being awarded to work determining the structure of biochemical substances including vitamin B_{12} and globular proteins, respectively. However, X-ray crystallography relies on forming a solid, crystalline sample, often technically challenging and not always representative of the environment in which a compound exists *in vivo*. It was not until advancements in nuclear magnetic resonance (NMR) in the 1970s that it was possible to capture information on the structure of molecules in solution.

The underlying physics of nuclear magnetic resonance (NMR) spectroscopy is more widely appreciated in the form of magnetic resonance imaging (MRI), a technique used in hospitals to image organs and diagnose diseases. In chemistry, rather than identifying the type of tissue, NMR can be used to identify the chemical groups found in a molecule and their relative connectivities. NMR signals can be translated into chemical structures for a wide variety of molecules, from small molecules to large proteins, either as solids or liquids.

Professor Anthony Serianni at the University of Notre Dame is an expert in utilising modern NMR techniques and computational approaches to identify the structures and reactivities of carbohydrates and nucleic acids. One of his approaches is to use 'isotopic labelling' to make it easier to differentiate between different regions of these large complex biomolecules. This technique has been so successful that it has been commercialised by his company, Omicron Biochemicals Inc., which provides services to researchers around the world.



(A) Structure of a Man_6 hexasaccharide containing eight sites of ^{13}C enrichment (♦) chosen to optimise the measurement of redundant *J*-couplings across its constituent *O*-glycosidic linkages. (B) Structure of a mature high-mannose *N*-glycan, $Man_9GlcNAc_2$, appended to protein. The subfragment highlighted in red corresponds to the structure in (A).

Professor Anthony Serianni at the University of Notre Dame is an expert in utilising modern isotope-based NMR techniques and computational approaches

SWEET STRUCTURES

Saccharides are a large family of molecules, including sugars, starch and cellulose, made mostly from carbon, oxygen and hydrogen. Saccharides are also known as carbohydrates and are ubiquitous in the biological world. Understanding their chemical structures is often key to understanding how they will bind and interact with the receptors in the human body, shaped in such a way that only molecules with complementary structures are able to fit.

The highly complex structures of many saccharides make standard NMR techniques very challenging to use. Professor Serianni's group has found a way to overcome this limitation by developing a range of new methods capable of introducing isotopic labels into specific sites in the compounds. An isotope is a version of a chemical element that has a different number of neutrons that, in an NMR experiment, acts as a unique flag for the labelled element in the compound. This is very powerful when looking at chemical reactions, as it is possible to follow the position of the label during the reaction to identify the structures of intermediates and end-products.

BIG BUSINESS

While introducing isotopic labels is a powerful tool to gain more structural information on molecules by NMR, introducing isotopes is a challenging problem, spanning both synthetic and biological chemistry. Fortunately, Professor Serianni and his team have developed a suite of methods that

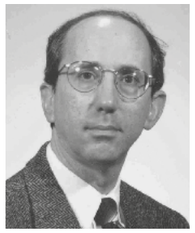
The University of Notre Dame and Omicron Inc. work in parallel, utilising complementary approaches to push the limits of isotopic labelling and the applications of labelling to solving important problems in chemistry and biochemistry

have transformed the possibilities for the types of compounds that can be precisely isotopically labelled at single sites, multiple sites, or uniformly labelled.

These approaches are currently being used to tackle fundamental chemical problems in the Notre Dame laboratory – the same research location that led to the development and expansion of Omicron Biochemicals, Inc. The work done in the Omicron research facility is somewhat different though, instead focusing exclusively on saccharide isotopic synthesis using chemical, biochemical and biological methods. This work has had an enormous impact worldwide, enabling other researchers to purchase isotopically-labelled compounds for use in their own work.

The labs at both Notre Dame and Omicron now work in parallel, utilising different approaches to push the limits of isotopic labelling and the applications of labelled saccharides to address chemical, biochemical and biomedical problems. Without this synergistic effort, tackling fundamental problems encountered in saccharide isotope labelling would have been difficult, and the core technologies underpinning Omicron would never have been developed or would have been developed more slowly.

In the future, Professor Serianni is optimistic that the innovative approaches pioneered in the Notre Dame Lab will lead to new spin-out companies able to exploit these findings and apply them to solve specific problems that impact human health and well-being.



Behind the Bench

Professor Anthony Serianni

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Research Objectives
Prof Serianni's research interests include methods development for site-specific labelling of carbohydrates, conformational studies of simple and complex carbohydrates related to the N-glycans of human glycoproteins by nuclear magnetic resonance, applications of molecular orbital theory to aid in the interpretation of NMR parameters, and structure-function studies of non-enzymic protein glycation.

Funding
National Science Foundation USA (NSF USA)

Collaborators
- Ian Carmichael (University of Notre Dame)
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- Paul Bondo (Omicron)
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Bio
Anthony Serianni received a BS in Biochemistry from Albright College, PA. He pursued graduate studies at Michigan State University, earning a PhD in 1980. He later moved to Cornell University for postdoctoral training, and in 1982, joined the University of Notre Dame, where he is currently Professor of Chemistry and Biochemistry.

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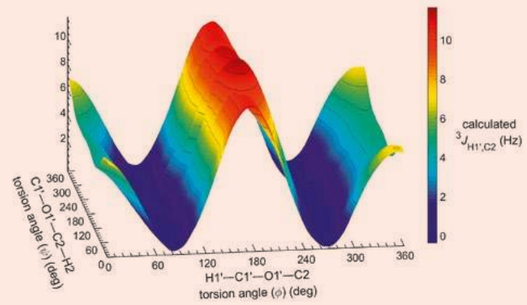
Q&A

What are the most important molecular structures you have been able to elucidate using your techniques?
We have focused our attention on the multiple structural elements in saccharides that collectively comprise their structures. The motivation is that, if we can develop more quantitative experimental tools to characterise these elements in solution, these tools can be applied generally to any structure, that is, we seek general applicability and thus greater impact. The research questions are fundamental so that their potential impact can be broad. Specifically, we aim to develop more detailed NMR-based models of many of the key mobile conformational elements found in saccharides, which include: (a) *O*-glycosidic linkages in oligosaccharides; (b) exocyclic hydroxymethyl group conformation; (c) *N*-acetyl side-chain conformation; (d) *O*-acetyl side-chain conformation; (e) hydroxyl group conformation; and (f) furanose and pyranose ring conformation. We have been frustrated with conventional NOE-based and simple *J*-based methods to characterise these behaviours because they often lead to generally unsatisfying solutions. This situation has led to an over-reliance on MD simulation and related methods to assess these behaviours, yet experimental validation of MD is weak. We contend that a more holistic treatment of NMR *J*-couplings, which are highly abundant in saccharides, is a potential solution to this problem, and recent studies using circular statistics in conjunction with DFT appear to provide conformational models that can be compared directly to those derived from MD. One of our core research goals is to parameterise all biologically relevant *O*-glycosidic linkages to enable studies of their conformational behaviours in simple and complex structures. This work hinges on the ability to label target molecules with ^{13}C at one or more sites to allow measurements of J_{CC} values. Recent expansions of this approach to studies of furanosyl rings, such as those found in DNA and RNA, appear promising; this work transcends typical J_{HH} analyses, as first applied by Altona, Sundaralingam and others, and offers the potential to characterise their conformational equilibria in greater detail and with greater reliability than has been possible for the past 40 years.

What are the big challenges ahead in 3D structural determination?
The assembly of larger oligosaccharides containing site-specific or multiple labelling with ^{13}C and/or other biologically relevant isotopes presents significant challenges (in contrast, uniform ^{13}C labelling is currently reasonably easy to accomplish using biological methods but often leads to NMR spectral complexity that resists analysis). Unlike oligopeptide and oligonucleotide synthesis, an automated instrument does not yet exist in the commercial sector to achieve assembly in high yields, although people such as Seeberger, Wong, Demchenko and others have been working to solve this problem. In addition, having the ability to incorporate labelled oligosaccharides into proteins (the latter either labelled or unlabelled) to generate chemically pure glycoproteins is still a major challenge; this problem needs to be solved if we want to fully exploit heteronuclear NMR and other methods in conjunction with stable isotopes to interrogate their structures, conformational features, time-dependent motions (dynamics), and biological functions.

What are some of the advantages of using NMR for structural determination?
Nowadays, techniques such as mass spectrometry (MS) have taken some of the wind out of NMR, being far more sensitive and thus amenable to determinations of primary saccharide structure when sample amounts are limited. Unlike for proteins, X-ray crystallography has not proven as generally useful for saccharide structure work because obtaining high quality crystals of saccharides, especially oligosaccharides, has proven to be challenging and unpredictable. Neither MS nor crystallography, however, provide information on saccharide solution behaviour, the biologically relevant state in many cases, and in this regard NMR still reigns supreme. Information on solution conformational equilibria and dynamics evolves from quantitative studies of different types of NMR parameters, giving for example a wealth of data on motions occurring on different time scales. These equilibria and dynamics properties are often closely associated with biological function.



A hypersurface showing the torsional dependencies of the trans-glycoside *J*-coupling, $^3J_{H1',C2}$, as determined by DFT for an αMan-(1→2)-αMan linkage like that between residues 5′ and 6′ in the hexasaccharide shown in Scheme 1 (A). This $^3J_{COCH}$ value depends primarily on the H1′–C1′–O1′–C2 torsion angle (φ), with only a minor dependence on the C1′–O1′–C2–H2 torsion angle (ψ).

Did you face any difficulties creating this spin-out company from your fundamental university-based research?
Omicron Biochemicals Inc. was founded in 1982 at a time when faculty-inspired companies were less common and less accepted in academic circles than they are today. In practice, founding and sustaining a company while functioning largely as a faculty member has proven challenging not in a technical sense, but rather in a social-psychological sense – the age-old question of which master do you serve? It is important to answer this question clearly, to be self-aware and cognizant of the importance of answering this question honestly. Omicron was founded for two main reasons: (1) there was a void in the scientific community that the company filled by widening the range of compounds that could be labelled in an affordable manner and on scales that enabled diverse applications; and (2) the company serves as a key research resource for academic studies at Notre Dame that require access to labelled compounds, without which the projects would be difficult if not impossible to undertake in a timely manner. Any success I may have achieved in academic research I attribute in significant measure to the unique opportunities offered by Omicron as a partner in the work.

Do you think it will become more common in the future to see spin-out companies developing from university research groups?
Yes, I do. Science, or specifically the conduct of science, is becoming increasingly codified. There is significant outsourcing of scientific work in most labs, and this trend is likely to continue over time. If you need to express a protein, you hire a company to do it. Scientific "kits" can be purchased to expedite specific kinds of lab work that would have taken considerable time and treasure previously. Perhaps equally important is that doing basic research might be less costly in a company environment than in an academic one, indirect costs being what they are. In the future, I would not be surprised if federal funding agencies increase their research support for companies engaging not only in applied research, but also in research at the fundamental level. Should this come to pass, academic institutions will need to redefine their relationships and regulations with respect to faculty entrepreneurs to ensure that the core values of both entities are protected and potential negative impacts on these values minimised.